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Evolution of MHC class I genes in two ancient fish, paddlefish (*Polyodon spathula*) and Chinese sturgeon (*Acipenser sinensis*)

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ABSTRACT

Here we present the first isolation of major histocompatibility complex (MHC) class I genes from two ancient fish, paddlefish (*Polyodon spathula*) and Chinese sturgeon (*Acipenser sinensis*). Seventeen sequences obtained showed high polymorphism and positive natural selection with $d_N/d_S > 1$. Evolutionary relationships revealed that sequences from paddlefish and Chinese sturgeon distinguished from other vertebrate class I and had an intermingling of alleles, which indicates that Acipenseriformes have a common ancestral gene of class I and a trans-species polymorphism across Acipenseriformes. We also found clear evidence of recombination among class I genes of paddlefish and Chinese sturgeon.

1. Introduction

The genes of the major histocompatibility complex (MHC) encode cell-surface glycoproteins that present peptides to T lymphocytes during an adaptive immune response in jawed vertebrates [1]. The MHC genes encode two major subfamilies of molecules, MHC class I and class II, which are functionally and structurally different. Class II molecules are heterodimers consisting of an α chain and a β chain, which are encoded by class II A and B genes, respectively. MHC class II molecules are only expressed on certain cells, such as B lymphocytes or macrophages, where they recognize and present extracellular peptides to helper T lymphocytes. MHC class I molecules, which are expressed on all nucleated somatic cells, recognize and present endogenous peptides to cytotoxic T lymphocytes. Class I molecules are heterodimers consisting of a heavy chain or α chain, encoded by class I genes, and called β2-microglobulin, encoded outside the MHC complex [2]. Class I genes comprise classical (class Ia) and non-classical (class Ib) loci which differ in polymorphism, structure, function and expression pattern.

MHC genes have been characterized in many vertebrate taxa, including mammals, birds, amphibians, reptiles and fishes. Despite conserved structures and functions, MHC gene evolution in teleosts differs from other vertebrate taxa. For example, class I gene loci are linked to class II gene loci in cartilaginous fish [3] and tetrapods, whereas in all teleost species studied, including the zebrafish, carp, salmon, trout and stickleback, class I and class II genes are not linked [4]. The "non-linkage" of these genes in teleosts may have occurred by differential silencing of MHC genes after genome-wide chromosomal duplication events [1] or by translocation that expelled some MHC genes to another chromosomal region [5,6]. In salmonid fish, the alleles of MHC class I genes comprise of several highly divergent lineages, whereas the alleles of class II genes are highly species specific, contrasting to the patterns in primates [7]. The prevalent recombination events also differ between MHC class I genes in teleosts and mammals. The intragenic recombination process involves entire exons in fish compared with much smaller fragments in humans. It is presumed that these different patterns of gene evolution might be due to the non-linkage of class I and class II loci in bony fish [7]. However, the study of MHC class I genes in the African clawed frog showed similar evolutionary patterns to salmonids, suggesting that non-linkage of class I and class II loci alone is insufficient to explain the patterns of MHC evolution

Abbreviations: MHC, major histocompatibility complex; Mya, million years ago; RNA, ribonucleic acid; DNA, deoxyribonucleic acid; cDNA, complementary DNA; PCR, polymerase chain reaction; RACE, rapid amplification of cDNA ends; Tm/cyt, transmembrane and cytosolic; PBR, peptide binding region

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in salmonids [8]. The ancient fish group, Neopterygii (gar and bowfin) and Chondrostei (bichir, paddlefish and sturgeon) occupy the most basal positions below the teleosts in Actinopterygii [9], which are the key lineages to reconstructing these events because of their unique evolutionary positions between tetrapods to teleosts [6]. Of the group, the Order Acipenseriformes is a particularly good model in which to study MHC gene evolution because of its unique evolutionary position and multiple diploid and polyploidy species. To date, no information has been reported on the MHC genes of the Neopterygii and Chondrostei.

Here, we isolated MHC class I genes from two species of Acipenseriformes, paddlefish, *Polyodon spathula* (Acipenseriformes: Polyodontidae) and Chinese sturgeon, *Acipenser sinensis* (Acipenseriformes: Acipenseridae), and examined the evolutionary patterns of the alleles and compared the results to the findings in other vertebrates. Paddlefish were distributed predominantly in North America but have since been introduced as commercial fish into many countries throughout the world. Chinese sturgeon is distributed mainly in the East China Sea and the Yangtze River in China. These two species represent major groups of the Acipenseriformes and diverged from each other around 184 million years ago (Mya) [10]. This study will help to elucidate the evolutionary history of MHC and will contribute towards understanding the immunology and conservation of paddlefish and sturgeons.

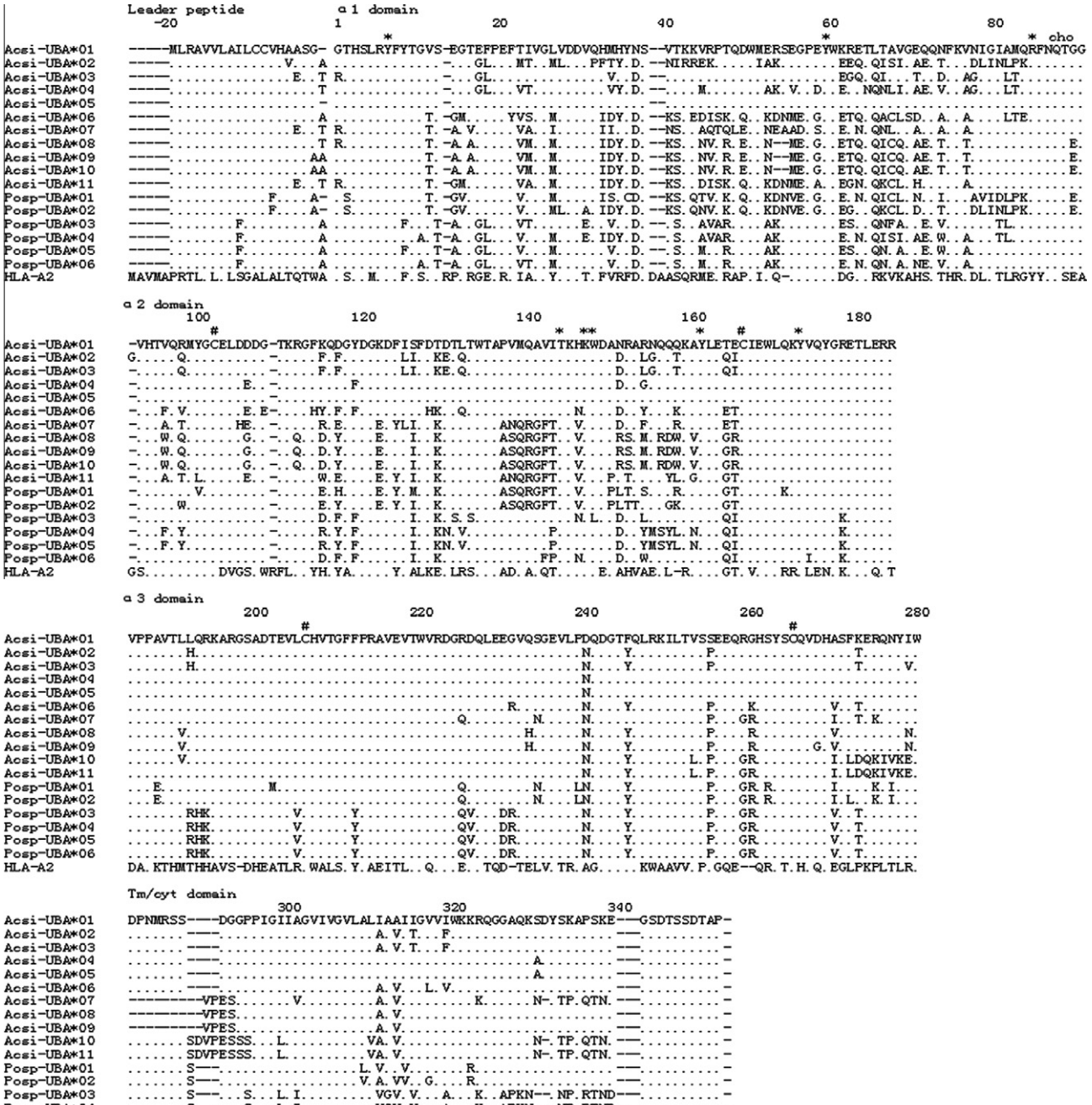

Fig. 1. Amino acid alignment of paddlefish and Chinese sturgeon MHC Class I with human HLA-A2 (*Homo sapiens*, K02883). Dots indicate identity with the *Acsi-UBA*01* sequence; dashes indicate gaps. Residues of interest are indicated above the sequence as: *, conserved sites for peptide binding; *cho*, glycosylation site; #, conserved cysteine residues. Nucleotide sequences are shown in Supplementary Fig. S1.

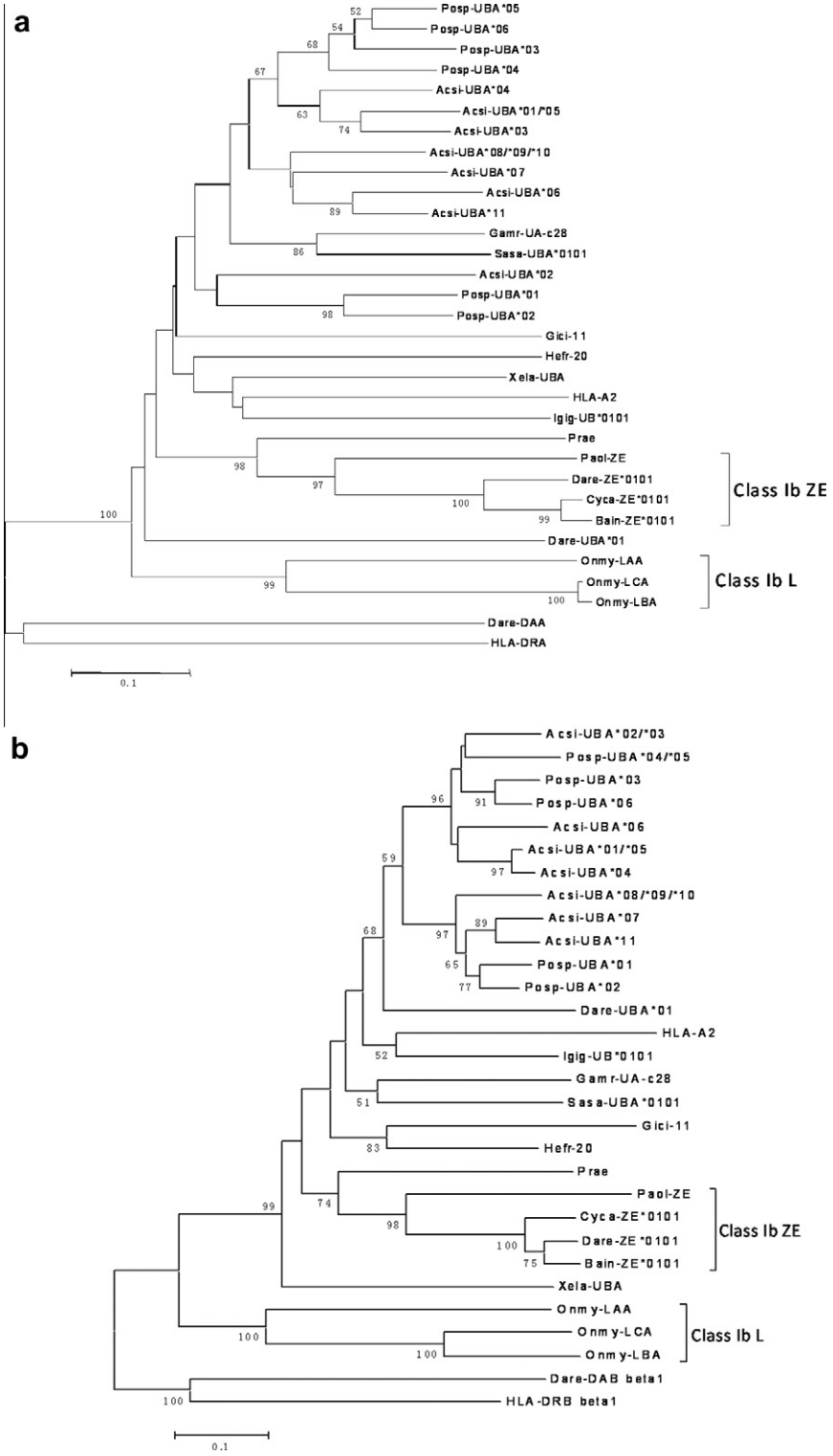

Fig. 2. Neighbor-Joining (NJ) phylogenetic trees reconstructed respectively by deduced amino acid of α1 (a), α2 (b), α3 (c) domains and full length (d) of vertebrate MHC class I. Sequences included are Gici-11 (nurse shark, *Ginglymostoma cirratum*, GenBank Accession: AF028557), Hefr-20 (horned shark, *Heterodontus francisci*, AF028559), Prae (marbled lungfish, *Protopterus aethiopicus*, AF206309), Xela-UBA (African clawed frog, *Xenopus laevis*, L20733), Igig-UB*0101 (common iguana, *Iguana iguana*, EU604317), HLA-A2, Dare-UBA*01 (zebrafish, *Danio rerio*, Z46777), Gamr-UA-c28 (Atlantic cod, *Gadus morhua*, AJ132511), Sasa- UBA*0101 (Atlantic salmon, *Salmo salar*, AF504019), Onmy-LAA (rainbow trout, *Oncorhynchus mykiss*, DQ789363), Onmy-LBA (*O.mykiss*, DQ789363), Onmy-LCA (*O.mykiss*, DQ789366), Paol-ZE (Japanese flounder, *Paralichthys olivaceus*, AB126916), Dare-ZE*0101 (*D.rerio*, AJ420953), Cyca-ZE*0101 (common carp, *Cyprinus carpio*, AJ420957) and Bain-ZE*0101 (large barbus, *Labeobarbus intermedius*, AJ420957). The latter seven sequences are L (Onmy-LAA, Onmy-LBA and Onmy-LCA) and Z/ZE (Paol-ZE, Dare-ZE*0101, Cyca-ZE*0101 and Bain-ZE*0101) lineages of non-classical class I genes of teleosts [12,13]. In α1 tree (a), α1 domain of class II α chain of zebrafish (Dare-DAA, L19446) and human (HLA-DRA, AK313123) were chosen as outgroup, and β1 and β2 domain of class II β chain of zebrafish (Dare-DAB, AY103492) and human (HLA-DRB, BC106057) as outgroup in α2 (b) and α3 (c) trees respectively. The Gici-11 and Hefr-20 were used as outgroup and non-classical class I genes were excluded in full length tree (d). Bootstrap values above 50% from 1000 replications were shown on nodes.

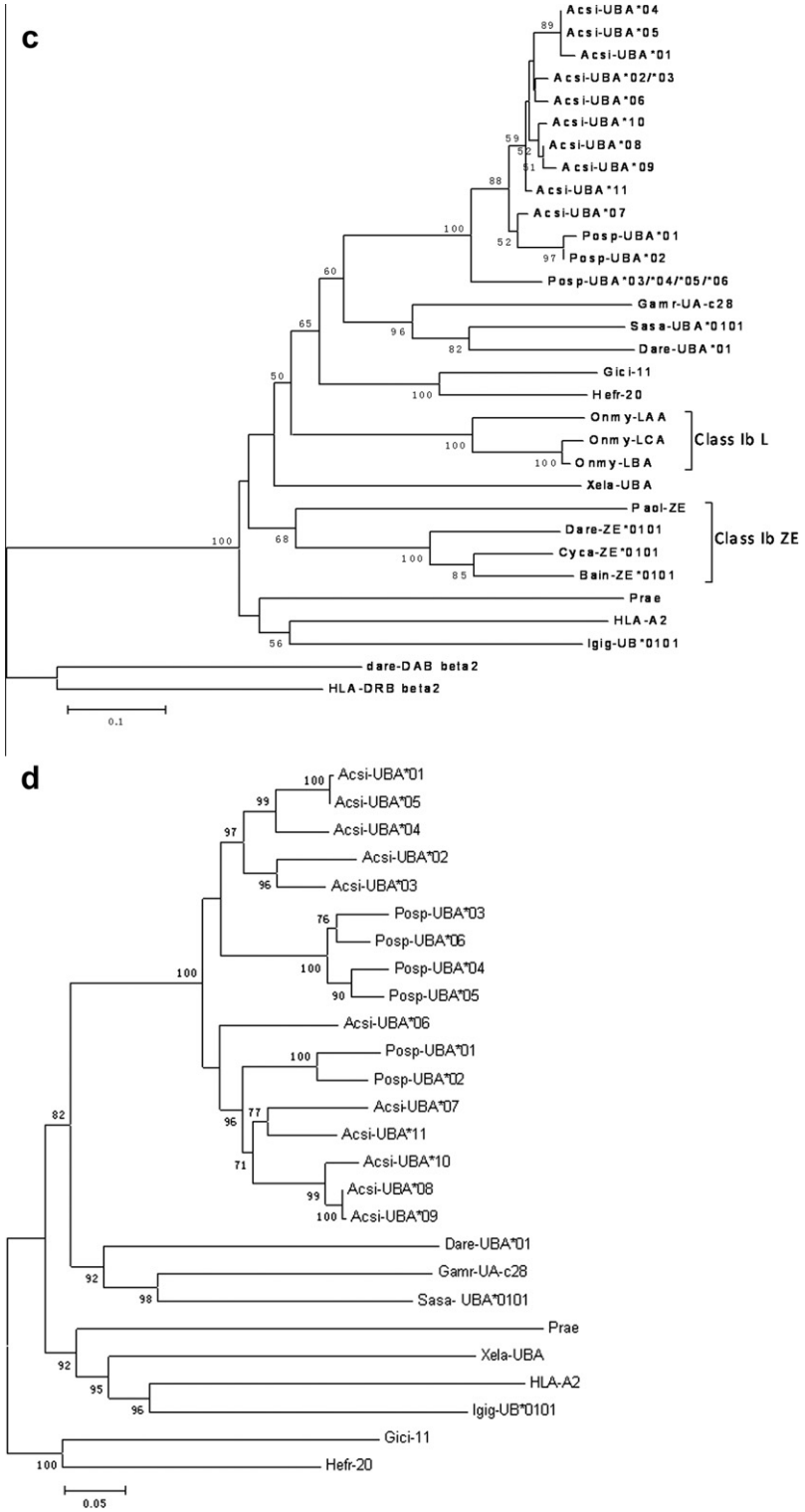

Fig. 2 (*continued*)

2. Materials and methods

2.1. RNA extraction and cDNA synthesis

Five paddlefish and five Chinese sturgeon were used in this study ribonucleic acid (RNA) was extracted from the spleen and the kidney using the RNAiso kit (TaKaRa, Dalian, China) and was treated with DnaseI to remove any genomic deoxyribonucleic acid (DNA). First strand complementary DNA (cDNA) was synthesized from 2 μg of total RNA using M-MLV Reverse Transcriptase (Promega, Shanghai, China) and Oligo$(dT)_{18}$.

2.2. MHC class I gene isolation

Fragments of MHC class I genes were amplified by polymerase chain reaction (PCR) with the primers MhcI F and MhcI R (extended

primers of Xia et al. [11]; Supplementary Table S1) from one fish of each species. The cDNA synthesized above was used as template. PCR included an initial denaturation step at 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, 54 °C for 30 s, and 72 °C for 60 s. PCR products were purified and then cloned into the pMD18-T vector (TaKaRa) and sequenced.

To obtain complete class I gene cDNA, we used the rapid amplification of cDNA ends (RACE) method following the user manual of SMART RACE cDNA Amplification kit (BD Biosciences Clontech, Germany). The above sequences were used to design gene-specific primers for 3′ RACE and 5′ RACE (Supplementary Table S1). Because of the high variability existing in overlapping regions, the sequences could not be exactly assembled for 3′ RACE to 5′ RACE. To obtain real allele sequences, an additional pair of primers, ACIF and ACIR (Supplementary Table S1), which anneal to the start and the end of the coding sequences, were designed to amplify nearly the entire MHC class I gene coding sequences of paddlefish and Chinese sturgeon. All of the sampled fishes were used in this step. The cDNA synthesis, PCR and cloning of each individual fish were performed independently twice, and 10–15 clones per individual and per PCR were sequenced. Only sequences identified in both PCRs for each fish were validated to avoid PCR artifacts.

2.3. Sequence analysis

Sequences were assembled and edited using the program Lasergene version 7 (DNAStar, http://www.dnastar.com/), and the amino acid sequences were aligned using the Clustal W software in MEGA version 4.0 [12]. The alignment files of α1, α2 and α3 domains were provided in Supplementary data. The non-synonymous/synonymous rate ratio ($\omega = d_N/d_S$) was used to test for selective pressure, and maximum likelihood (ML) ratio tests in CODEML were performed to compare model M7 with M8 [13]. Phylogenetic relationships among the MHC class I genes of paddlefish, Chinese sturgeon and other vertebrates, including the class Ib teleosts [14,15] (Figs. 1 and 2), were reconstructed for α1, α2 and α3 sequences separately. This partitioning method can reflect the evolutionary history of these functionally distinct regions. Phylogenetic trees were constructed with the program MEGA version 4.0, using a *p*-distance model for multiple substitutions and the neighbor-joining method. To deal with the evolutionary relationship involved in gene duplication and recombination, a Neighbor-Net method might be more appropriate comparing phylogenetic trees [16]. We constructed phylogenetic networks for each α domain and combined all domains using the program SplitsTree4 [17].

Recombination analyses were performed using the program RDP 3 beta 36 package [18]. The highest acceptable P value inferring recombination was set at 0.001 with a window size of 20 nucleotides (nt). Four methods, including RDP, GENECONV, Maximum Chi and BootScan, were used to detect recombination signals and recombinant sequences. Only recombination events that were confirmed by all methods were considered to be true. To estimate the recombination rates (Rho = 4Ner) and the mutation rates (theta = 4Neμ) along the MHC class I gene, we used the INTERVAL program [19] with 10 as a starting Rho, implemented in the RDP package.

3. Results

3.1. Sequence characterization

Two sequences from 3′RACE and two sequences from 5′RACE from paddlefish, and three sequences from 3′RACE and one sequence from 5′RACE from Chinese sturgeon (GenBank Accession Nos.: GQ485558–GQ485565) were obtained. Using primers ACIF and ACIR, the nearly complete coding regions of six alleles from paddlefish and 11 alleles from Chinese sturgeon were identified, which were designated as *Posp-UBA*01–Posp-UBA*06* and *Acsi-UBA*01–Acsi-UBA*11* (GenBank Accession Nos.: GQ485566–GQ485582), respectively. These alleles were used for further analysis.

Both the paddlefish and Chinese sturgeon MHC class I UBA had an α1 domain of 88 codons, an α2 domain of 91 codons and an α3 domain of 96 codons, except for a deletion of two codons in the α1 domain for *Acsi-UBA*08, -*09* and *-*10* and an insertion of one codon in the α1 domain for *Acsi-UBA*01*. There was an indel (insertion/deletion) of one codon in the leader domain and four indels of one to nine codons in the transmembrane and cytosolic (Tm/cyt) domains, as shown in the alignment of these sequences (Fig. 1; Supplementary Fig. S1). The pairwise nucleotide *p*-distances for the α1, α2 and α3 domains were 4.5–31.1% (mean: 19.3%), 0–23.4% (14.6%) and 0–6.6% (3.8%), respectively, in the paddlefish sequences, and 0–29.1% (21.3%), 0–20.5% (13.6%) and 0.03–6.6% (5.0%), respectively, in the Chinese sturgeon sequences (Supplementary Tables S2 and S3). The d_N/d_S ratios of the α1 and α2 domains were >1 with the M8 model in both fish, suggesting that positive selection is acting on this region (Table 1). Twenty-six sites were identified as being under positive selection ($P > 0.99$) in the Chinese sturgeon sequences. Twenty-four of these sites resided in the α1 and α2 domains, 20 of which were consistent with the peptide binding site of human class I MHC [20]. The remaining two sites resided in the α3 domain, similarly to that reported for the cattle class I MHC [21]. Fifteen sites were identified as being under positive selection ($P > 0.99$) in paddlefish sequences, all except two of which were consistent with those identified in Chinese sturgeon.

All of the protein sequences showed the typical features of classical MHC class I α chains when they were aligned with human HLA-A2 protein sequences (Fig. 1). There were two sets of cysteines in the α2 (positions 102 and 166) and α3 (positions 206 and 265) domains which likely form intrachain disulfide bonds. A potential N-glycosylation site (NQT) was also identified at positions 87–89 in the α2 domain (Fig. 1). Eight sites in the peptide binding region (PBR) protein of non-mammalian vertebrates have been identified as important anchor points for peptide binding [14], all of which were conserved in paddlefish and Chinese sturgeon MHC class I molecules, expect for position 148 encoded by *Posp-UBA*03*.

Table 1
Selection test of MHC class I in Chinese sturgeon and paddlefish.

	Model	Log likelihood	d_N/d_S	Estimates of parameters	Sites[a] under positive selection ($P > 0.99$)
Chinese sturgeon	M7 (beta)	−2964.51	0.407	$p = 0.091$, $q = 0.131$	Not allowed
	M8 (beta & ω)	−2881.59	1.548	$p_0 = 0.828$, ($p_1 = 0.171$)	24, 33, 34, 41, 45, 46, 63, 67, 68, 69, 71, 74, 82,
				$p = 0.020$, $q = 0.027$, $\omega = 7.049$	96, 98, 115, 117, 118, 146, 151, 154, 158, 164, 165, 273, 279,
Paddlefish	M7 (beta)	−1783.64	0.516	$p = 0.023$, $q = 0.020$	Not allowed
	M8 (beta & ω)	−1728.35	3.410	$p_0 = 0.873$ ($p_1 = 0.127$)	34, 45, 46, 63, 68, 71, 74, 82L, 115, 117, 154, 156, 157, 158
				$p = 0.007$, $q = 0.005$, $\omega = 19.453$	

[a] Sites as Fig. 1.

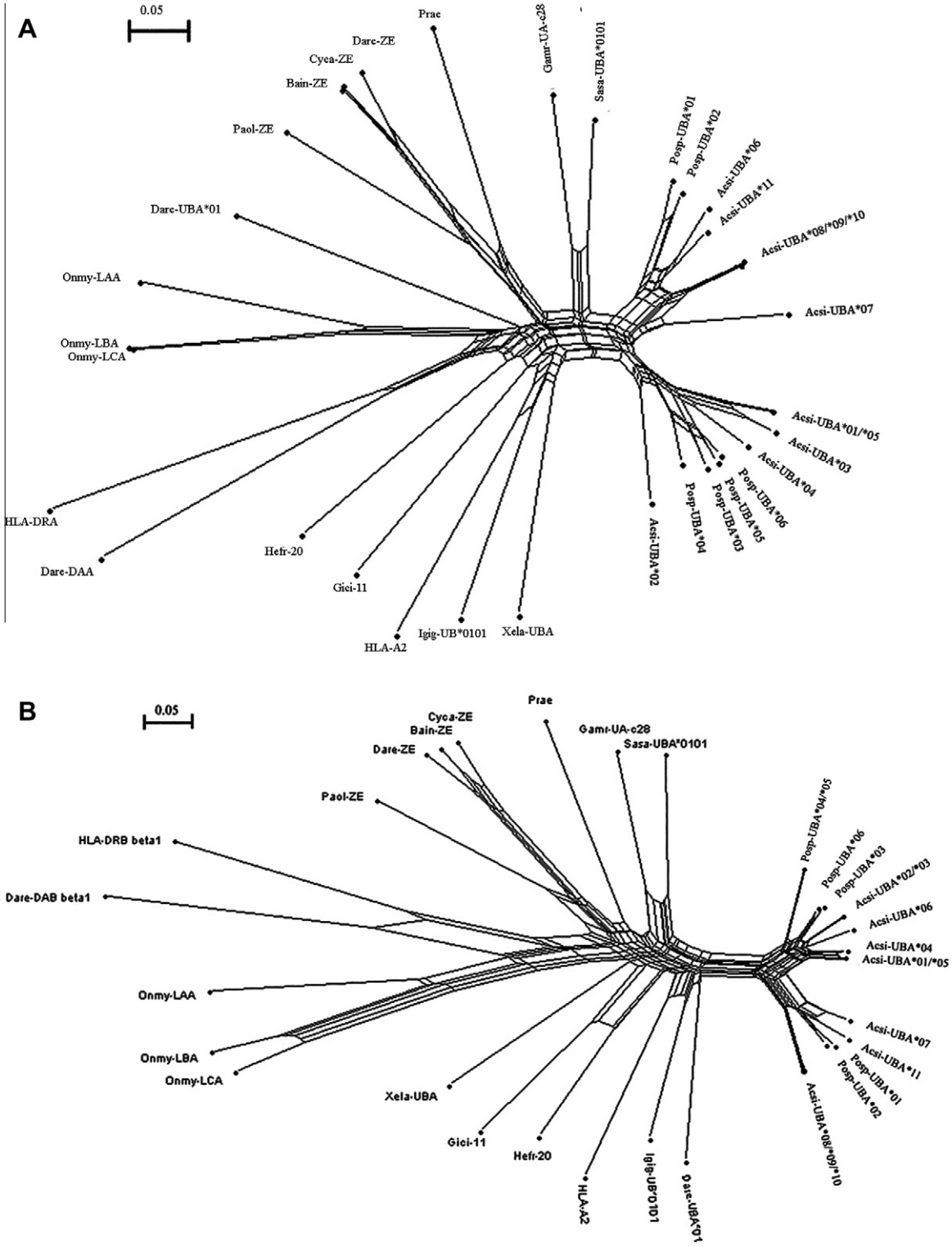

Fig. 3. Networks generated from α1 (A), α2 (B) and α3 (C) domains of vertebrate MHC class I. Sequences used here are the same as Fig. 2. The Networks were constructed in SplitsTree v4.0 with model of uncorrected p-distance.

3.2. Phylogenetic relationships

The topologies of the α1, α2 and α3 domains differed (Fig. 2), reflecting the distinct evolutionary pathways of these functionally distinct regions. In the α1 tree, the sequences of paddlefish/Chinese sturgeon and the class Ia sequences of Atlantic cod and Atlantic salmon clustered as a group, and the other teleost, zebrafish class Ia (Dare-UBA*01), was divergent from this cluster. However, Dare-UBA*01 was closer to the sequences of paddlefish/Chinese sturgeon, which clustered as a monophyletic family in the α2 tree. The teleost non-classical L lineage and the ZE lineage containing lungfish class I (Prae), were basal of all class I sequences in both the α1 and α2 trees, except for Dare-UBA*01 or Xela-UBA. Monophyly of sequences of paddlefish/Chinese sturgeon was supported

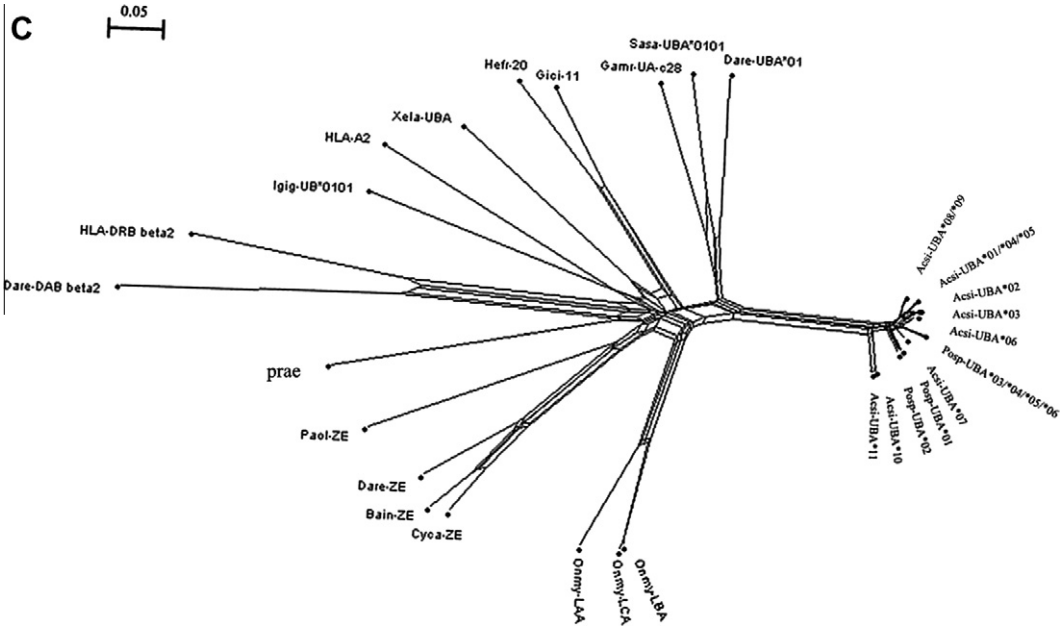

Fig. 3 (*continued*)

Table 2
Recombinant sequences at the paddlefish and Chinese sturgeon UBA loci.

Recombinant sequences	Nucleotide breakpoint	Potential parent sequences
*Acsi-UBA*06*	329	Acsi-UBA*11/Acsi-UBA*05
*Acsi-UBA*11*	nd	Posp-UBA*01/Unknown
*Posp-UBA*01*	332	Unknown/Acsi-UBA*11
*Posp-UBA*02*	265	Unknown/Acsi-UBA*09

Note: nd, not determined.

by 100% bootstrap values in the α3 tree, which was a sister of the teleost class Ia. No species-specific clusters for sequences of paddlefish/Chinese sturgeon were supported in any of the trees.

Phylogenetic networks (Fig. 3) constructed for each domain showed many conflicting signals, which suggested that recombination between sequences has occurred. The sequences of paddlefish and Chinese sturgeon formed two distinct clusters in the α1 and α2 networks (Fig. 3A and B), whereas they were not such constraints in the α3 networks (Fig. 3C; Supplementary Fig. S2).

3.3. Recombination test

Recombination plays an important role in MHC gene evolution of paddlefish and sturgeons. We set conservative parameters of RDP3 to avoid false-positive identification of recombination events, and obtained four recombinant sequences (Table 2), representing a minimum number of recombinant alleles. Three recombination breakpoints were determined, of which two were situated in the α2 domain and one in the α1 domain. Estimation of the mutation and recombination rates gave relatively high values. Recombination rate analysis along the sequence alignments showed considerable evidence of recombination across a region of about 189 bp between the α1 and α2 domains (Fig. 4).

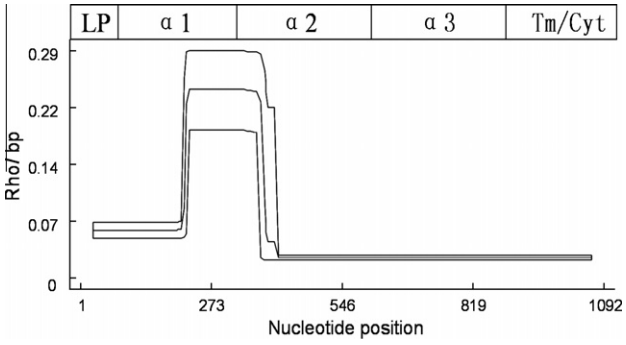

Fig. 4. Recombination rates (rho) estimated along the paddlefish and Chinese sturgeon MHC class I nucleotide sequence alignment (Supplementary Fig. S1). The mean rho (bold line) per bp and the upper and lower intervals (ashen lines) of 95 percent confidence were shown. The protrudent area displayed the region of highest rho corresponding to nucleotide sites 206–392 in the alignment as shown in Supplementary Fig. S1. Rho (=67.07)/theta (76.91) = 0.892.

4. Discussion

The relatively conserved α3 domain has been used to study the long-term evolution of class I MHC [6,14], and the topology of which revealed that sequences of paddlefish and sturgeon were closer to the classical class I genes rather than the non-classical class I genes of teleosts (Figs. 2 and 3). This suggested that the sequences isolated in this study represent classical class I genes. The evidence of positive selection on the α1 and α2 domains further supports this conclusion (Table 1). Phylogenetic networks constructed using the sequences of the α1, α2 and α3 domains and combined sequences, showed that the sequences of paddlefish and Chinese sturgeon clustered separately from other sequences (Fig. 3), demonstrating that class I genes of Acipenseriformes diverged from a common ancestral gene after the separation of Acipenseriformes from all other vertebrate lineages. The ancestral gene has repeatedly undergone gene duplication to produce multiple copies which then diverged from each other. Some of the duplicated genes are maintained in the genome, while others have been lost or become pseudogenes because of mutations. Such a pattern of MHC evolution is known as the birth-and-death model [22], and has also been seen in many other lineages [23,24,21,25,26]. The actual class I loci of both fish are still to be determined, however based on the fact that four alleles were isolated from a Chinese sturgeon and three from a paddlefish (Supplementary Table S4),

it is presumed that there are at least two class I loci expressed. For Chinese sturgeon, the alleles *Acsi*-UBA*01–*05 could be from a single locus due to the low variability in sequence among them, and the fact that they are clustered together in phylogenetic trees and networks. An indel of four codons in the Tm/cyt domain (Fig. 1; Supplementary Fig. S1) may be an indicator of this locus when the potential recombinant, *Acsi*-UBA*06, is not taken into account. The other sequences of Chinese sturgeon cannot be assumed to be from the same locus, because of inconsistencies in sequence and their unstable positions in trees and networks, especially in the α3 tree and network (Figs. 2 and 3). Chinese sturgeon may harbor more than two class I loci. Likewise, *Posp*-UBA*01 and *02 and *Posp*-UBA*03–*06 appear to belong to two different class I loci.

Though only two species were studied, there was evidence that the phylogenetic trees and networks (Figs. 2 and 3) showed mixing of alleles, indicating that trans-species polymorphisms occur across the Acipenseriformes because the two species represent the only two families in this order. This is supported by topologies of only d_S or the third condon position considered (data not shown). Fossil records [27] and molecular data [10] showed that the split time between paddlefishes and sturgeons could be dated back to more than 184 Mya. This implies that some allele lineages of MHC in Acipenseriformes have been maintained for up to 184 Mya. Trans-species polymorphisms in class I MHC had been observed in many related taxa, but could be confined to closely related species. For example, human HLA class I lineages are only recognized in great apes and thus have been maintained for 6 Mya [23]. Similarly, African clawed frog class I lineages may only be maintained in *Xenopus* [8]. Some class I lineages were reported to be maintained for a longer time in teleosts than in mammals. For example, salmonid class I UBA lineages were found to have been maintained for about 15–20 Mya [7], and a non-classical class I ZE or L lineage in cyprinids was estimated to last for up to 100 Mya [14,15]. Thus, the class I lineages in paddlefish and Chinese sturgeon are the oldest identified to date, which is remarkable, particularly when considering the classical class I MHC. The trans-species polymorphisms that have occurred during evolution in paddlefish and sturgeon must be strongly influenced by balancing selection acting on the class I locus.

Our analysis exhibited clear signals of recombination events that had occurred in MHC class I genes in paddlefish and Chinese sturgeon, with the recombination rates highest in the α1 and α2 domains (Fig. 4; Supplementary Fig. S1). Given that all of the determined breakpoints were located near to the connections between the α1 and α2 domains, which are usually separated by intron II, recombination is likely to have occurred in the intron leading to exchange of the entire exons. A bias in the α1 and α2 domains involved in recombination has been detected for some species, such as salmonid [7] and frog [8], which usually occurs in intron II, leading to "exon shuffling", creating new arrangements of the PBR. Recombination is believed to be an important factor generating patterns of reticulate evolution in phylogenetic trees reconstructed with different regions of MHC sequences in frogs, birds and humans [8,28,29], and this was confirmed by the results of our study (Fig. 4). Interlocus recombination may be a major mode of recombination in the class I MHC of paddlefish and sturgeons. In the case of *Acsi*-UBA*06, its potential parents, *Acsi*-UBA*11 and *Acsi*-UBA*05, were presumed to represent different loci, thus its position was unstable in relation to different domains. As a ubiquitous evolutionary force, recombination has been shown to contribute substantially to MHC diversity [30], creating new alleles in isolated populations [31]. The ratio of rho to theta was 0.892 (Fig. 4), suggesting that the rate of accumulation of new recombinants approaches that of new mutations.

In conclusion, MHC class I evolution in paddlefish and Chinese sturgeon is similar to MHC evolution in teleosts, except that class I lineages in paddlefish and Chinese sturgeon are older. In future research, more species of Acipenseriformes should be studied and class II genes should also be characterized.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.febslet.2010.05.065.

References

[1] Klein, J. (1986) Natural history of the major histocompatibility complex, Wiley, New York.
[2] Hughes, A.L. and Yeager, M. (1998) Natural selection at major histocompatibility complex loci of vertebrates. Annu. Rev. Genet. 32, 415–435.
[3] Ohta, Y., Okamura, K., McKinney, E.C., Bartl, S., Hashimoto, K. and Flajnik, M.F. (2000) Primitive synteny of vertebrate major histocompatibility complex class I and class II genes. Proc. Natl. Acad. Sci. USA 97, 4712–4717.
[4] Kelley, J., Walter, L. and Trowsdale, J. (2005) Comparative genomics of major histocompatibility complexes. Immunogenetics 56, 683–695.
[5] Kuroda, N., Figueroa, F., O'hUigin, C. and Klein, J. (2002) Evidence that the separation of Mhc class II from class I loci in the zebrafish, *Danio rerio*, occurred by translocation. Immunogenetics 54, 418–430.
[6] Stet, R.J., Kruiswijk, C.P. and Dixon, B. (2003) Major Histocompatibility lineages and immune gene function in teleost fishes: the road not taken. Crit. Rev. Immunol. 23, 441–471.
[7] Shum, B.P. et al. (2001) Modes of salmon MHC class I and II evolution differ from the primate paradigm. J. Immunol. 166, 3297–3308.
[8] Bos, D.H. and Waldman, B. (2006) Evolution by recombination and trans-species polymorphism in the MHC Class I gene of *Xenopus laevis*. Mol. Biol. Evol. 23, 137–143.
[9] Inoue, J.G., Miya, M., Tsukamoto, K. and Nishida, M. (2003) Basal actinopterygian relationships: a mitogenomic perspective on the phylogeny of the "ancient fish". Mol. Phylogenet. Evol. 26, 110–120.
[10] Peng, Z., Ludwig, A., Wang, D., Diogo, R., Wei, Q. and He, S. (2007) Age and biogeography of major clades in sturgeons and paddlefishes (Pisces: Acipenseriformes). Mol. Phylogenet. Evol. 42, 854–862.
[11] Xia, C., Xu, G., Lin, C., Hu, T., Yan, R. and Gao, G. (2004) Molecular cloning and polymorphism of major histocompatibility complex class I genes from grass carp (*Ctenophayngodon idellus*). Prog. Bio. Sci. 14, 403–410.
[12] Tamura, K., Dudley, J., Nei, M. and Kumar, S. (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24, 1596–1599.
[13] Yang, Z., Nielsen, R., Goldman, N. and Pedersen, A.M. (2000) Codon-substitution models for heterogeneous selection pressure at amino acid sites. Genetics 155, 431–449.
[14] Kruiswijk, C.P., Hermsen, T.T., Westphal, A.H., Savelkoul, H.F. and Stet, R.J. (2002) A novel functional class I lineage in zebrafish (*Danio rerio*), carp (*Cyprinus carpio*), and large barbus (*Barbus intermedius*) showing an unusual conservation of the peptide binding domains. J. Immunol. 169, 1936–1947.
[15] Dijkstra, J.M. et al. (2007) A third broad lineage of major histocompatibility complex (MHC) class I in teleost fish; MHC class II linkage and processed genes. Immunogenetics 59, 305–321.
[16] Huson, D.H. and Bryant, D. (2006) Application of Phylogenetic networks in evolutionary studies. Mol. Biol. Evol. 23, 254–267.
[17] Bryant, D. and Moulton, V. (2004) Neighbor-net: an agglomerative method for the construction of phylogenetic networks. Mol. Biol. Evol. 21, 255–265.
[18] Martin, D.P., Williamson, C. and Posada, D. (2005) RDP2: recombination detection and analysis from sequence alignments. Bioinformatics 21, 260–262.
[19] McVean, G., Awadalla, P. and Fearnhead, P. (2002) A coalescent-based method for detecting and estimating recombination from gene sequences. Genetics 160, 1231–1241.
[20] Parham, P., Adams, E.J. and Arnett, K. (1995) The origins of HLA-A, B, C polymorphism. Immunol. Rev. 143, 140–180.
[21] Holmes, E.C., Roberts, A.F., Staines, K.A. and Ellis, S.A. (2003) Evolution of major histocompatibility complex class I genes in Cetartiodactyls. Immunogenetics 55, 193–202.
[22] Nei, M., Gu, X. and Sitnikova, T. (1997) Evolution by the birth-and-death process in multigene families of the vertebrate immune system. Proc. Natl. Acad. Sci. USA 94, 7799–7806.
[23] Vogel, T.U., Evans, D.T., Urvater, J.A., O'Connor, D.H., Hughes, A.L. and Watkins, D.I. (1999) Major histocompatibility complex class I genes in primates: co-evolution with pathogens. Immunol. Rev. 167, 327–337.

[24] Miller, K.M., Kaukinen, K.H. and Schulze, A.D. (2002) Expansion and contraction of major histocompatibility complex genes: a teleostean example. Immunogenetics 53, 941–963.
[25] Ottová, E., Simková, A., Martin, J.F., de Bellocq, J.G., Gelnar, M., Allienne, J.F. and Morand, S. (2005) Evolution and trans-species polymorphism of MHC class IIβ genes in cyprinid fish. Fish&Shellfish Immunol. 18, 199–222.
[26] Burri, R., Hirzel, H.N., Salamin, N., Roulin, A. and Fumagalli, L. (2008) Evolutionary patterns of MHC class II B in owls and their implications for the understanding of avian MHC evolution. Mol. Biol. Evol. 25, 1180–1191.
[27] Grande, L. and Bemis, W.E. (1991) Osteology and phylogenetic relationships of fossil and recent paddlefishes (Polyodontidae) with comments on the interrelationships of Acipenseriformes. J. Vertebr. Paleontol. 11 (Suppl. 1), 1–121.
[28] Wittzell, H., Bernot, A., Auffray, C. and Zoorob, R. (1999) Concerted evolution of two Mhc class II B loci in pheasants and domestic chickens. Mol. Biol. Evol. 16, 479–490.
[29] Jakobsen, I.B., Wilson, S.R. and Easteal, S. (1998) Patterns of reticulate evolution for the classical class I and II HLA loci. Immunogenetics 48, 312–323.
[30] Bergström, T.F., Josefsson, A., Erlich, H.A. and Gyllensten, U. (1998) Recent origin of HLA-DRB1 alleles and implications for human evolution. Nat. Genet. 18, 204–206.
[31] Consuegra, S., Megens, H.J., Schaschl, H., Leon, K., Stet, R.J. and Jordan, W.C. (2005) Rapid evolution of the MH class I locus results in different allelic compositions in recently diverged populations of Atlantic salmon. Mol. Biol. Evol. 22, 1095–1106.